

Quarterly Report to NH DES

Evaluation of Seven Aquatic Herbicides for Selective Control of Variable Milfoil (*Myriophyllum heterophyllum* Michx)

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Plant Collection and Culturing:

Variable milfoil was collected by NHDES personnel from several sites within the State of New Hampshire (Table 1.) and sent to the University of Florida, Center for Aquatic and Invasive Plants (CAIP), in Gainesville, Florida.

Table 1. Variable milfoil collection sites.

Site	Size (Ha)	County	Sites Sampled
Horseshoe Pond	15.1	Hillsborough	3
Lees Pond	72.5	Carroll	3
Turkey Pond	65.8	Merrimack	3
Lake Winnepesaukee (Wolfe Bay)	18,043	Belknap	1
Lake Massabesic	1173.6	Rockingham	1

Healthy apical tips from these samples were excised and planted into 3 Liter pots that contained either an organic sediment (41% organic, 44% silt, 13% clay) collected from Bivens Arm Lake, FL, Vitahume potting soil (80% sand, 11% silt, and 8% organic), a mixture of 50/50 Vitahume potting soil and builders sand, or a 50/50 mixture of the potting soil and organic sediment from Bivens Arm. All of the sediments have been used in previous efforts at the CAIP to culture submersed plants. All sediment mixtures were amended with Osmocote 15:15:15 at a rate of 2 g/Kg of dry sediment. Plants from different collection sites were grown in separate 900 L concrete mesocosm tanks, and each sediment type was labeled. Culture water was amended to achieve an initial pH of ~6.5, conductivity of ~100 umhos/cm, and alkalinity (A.N.C) between 5 and 15. Cultures were maintained under ambient temperatures with the exception of using a large chiller that circulated cold water (12 C) through a piping system during the warmest months.

Results:

Variable milfoil grew well under the culture conditions and plants rapidly established on all of the sediments. Clippings of 10 to 12 cm, readily took root in the sediment and shoots grew to the water surface (65 cm) and formed dense canopies within 1 month. Initial harvests suggested that VM grew the fastest in the 50/50 potting soil and muck mixture; however, the plants growing in potting soil alone showed the best long-term culture properties. For our cultures, it is important that the plants can withstand

numerous clippings of apical meristems. Based on observations of the recovery potential following meristem removal, all subsequent cultures will rely on the use of potting soil with an Osmocote amendment.

We have noted that some cultures are prone to developing dense epiphytic growth over time. The switch from a clean macrophyte system to one that contains macrophytes and associated epiphytes can be quite rapid. These epiphyte blooms tend to be persistent, and we have found that re-culturing the plants is the best method for restoring clean cultures. Aside from potential studies evaluating the impacts of epiphytes on diquat efficacy, our study protocols call for the use of clean shoot meristems for herbicide efficacy studies.

Initial Herbicide Testing – Evaluation of Intra-site and Inter-site Variation:

Based on prior published work, we chose the herbicides 2,4-D, triclopyr, and diquat to conduct initial efficacy comparisons for evaluating intra-site and inter-site variation. VM from each collection site within the 5 sample lakes was exposed to 2,4-D and triclopyr at concentrations of 0, 1.0 and 2.0 mg/L for 2, 6, 12, and 24 hours. VM was exposed to diquat concentrations of 0, 0.15, and 0.30 mg/L for 1.5, 3, 6, and 18 hours. Following exposure, plants were given a 21-day post-treatment period. Plants were harvested at this time and total stem length and weight were recorded. Each treatment was replicated 4 times.

Results:

As expected, within a given sample site (e.g. Turkey pond Site 1) significant response differences were noted between the various concentration and exposure time scenarios for each individual herbicide tested. Nonetheless, there were no strong indications of intra-site or inter-site variation in response to 2,4-D, triclopyr, or diquat applications. Of eleven individual sample sites evaluated, we found significant differences in response to a given herbicide concentration and exposure treatment in only 7/132 treatments for 2,4-D, 10/132 treatments for triclopyr, and 5/132 treatments for diquat. No clear trends were noted for these differences, and given the limited number of differences detected, we conclude that our culture populations would be expected to respond to herbicide treatments in a similar manner. We have not directly compared the response between the herbicide treatments in our analysis (i.e. direct comparison of 2,4-D and triclopyr at similar rates and exposure); however, we can conduct this analysis on the dataset in the future.

Given the size of this study and time required to repeat it, we conducted a second trial using selected concentrations and exposure periods for the various herbicides. Triclopyr and 2,4-D were evaluated at 0 and 1.0 mg/L for 12 and 24 hours, and diquat was evaluated at 0 and 0.30 mg/L for 6 and 18 hours. The study included a total of 44 treatments for each sample site. Results of the second study confirmed the first, with differences between treatments noted in only 2/44 triclopyr treatments and 2/44 diquat treatments. No differences were noted for any of the 2,4-D treatments.

This testing did not take into account various physical and environmental parameters that can impact a treatment in the field (e.g. turbidity, sediment quality, plant phenology and stage of growth, treatment timing), but it does demonstrate that significant variation in response to herbicide treatments is not likely due to differences in plant collection site or biotype.

Based on the results of these studies we have chosen to maintain our VM cultures in separate tanks; however, future testing will not require an independent assessment of plants from each lake and sample site. This strategy will allow us to increase our focus on the response to herbicide treatments and reduce our focus on potential population differences.

Future work:

We have initiated pilot studies with fluridone to evaluate the potential for site-specific differences in response of VM to this herbicide. Protocols for evaluating response to fluridone and other slow acting herbicides vary significantly from the contact and auxin-type herbicides. These results will be reported in the March report.

Initial Efficacy Testing of Contact and Auxin-Type Herbicides :

We have currently identified 10 herbicides to be evaluated for activity on Variable Milfoil. These include the registered products carfentrazone, copper, diquat, endothall, 2,4-D, fluridone, and triclopyr. We also have 3 new compounds, penoxsulam, imazamox, and flumioxazin, which have recently received US EPA Experimental Use Permits. Based on our knowledge of recent industry activity, there are an additional two new compounds that may be submitted for Experimental Use Permits within the next 3 months. This would result in 12 potential compounds for evaluation. To date, we have chosen to focus on registered products, but feel that evaluation of the EUP products is warranted for several reasons. While this may require a slight alteration to the proposed schedule of work, the tradeoff is the generation of information on new classes of herbicides that would be available in the NHDES VM control program.

Research conducted to date has focused on the efficacy of carfentrazone, diquat, endothall, triclopyr, and the amine and ester formulations of 2,4-D. As noted above, for these evaluations we are not distinguishing the source of the plant material. Below, we provide a summary for each compound evaluated to date. Studies have been conducted only once and therefore each needs to be repeated prior to drawing more definitive conclusions.

Carfentrazone:

Based on the label rates and prior work conducted with other milfoil species, we designed studies to determine the efficacy of carfentrazone against VM. Carfentrazone is a protox inhibitor that has rapid contact activity. VM was exposed to carfentrazone at rates of 0, 50, 100, and 200 ppb for exposure periods of 0.5, 1, 3, 6, 12, and 24 hours. Plants were evaluated over a 21-day period and then harvested. Each treatment was replicated 5 times.

Following exposure, plants were noted to become brown within days of exposure. Results suggest that carfentrazone is very effective against VM at rates of 100 and 200 ppb at exposures of 1 hour and greater. The 50 ppb treatment was not effective at exposures of 3 hours and less; however, plants exposed for 6 hours and greater showed strong herbicide injury compared to untreated controls. These results were somewhat unexpected given the generally weak response demonstrated by other milfoil species. It should be noted that our exposures were conducted under a pH of ~6.5, while other milfoil species were likely exposed when the aqueous pH was much greater (8.0 to 9.0). This suggests that prevailing water quality conditions in New Hampshire may favor a compound such as carfentrazone that shows strong activity in lower pH waters. While results still need to be confirmed, data suggest that carfentrazone may have strong potential as a contact herbicide for control of VM. Carfentrazone was registered as a reduced risk herbicide, and given the specificity for a plant enzyme and short half-life in the water; this product has strong merit for further evaluation.

Diquat:

We suspected that diquat would be highly efficacious in our trials due to the fact that we work in “clean” systems that have minimal clay turbidity or particulate organic matter. In addition, the culture plants tend to be clean and free of epiphytes or seston. Prior research conducted with Eurasian milfoil showed diquat to be extremely efficacious in our clean systems. Diquat was evaluated at rates of 90, 135, 180, 270, and 370 ppb for exposure periods of 0.5, 1, 3, 6, 12, and 24 hours for VM control. Plants were evaluated over a 21-day period and then harvested. Each treatment was replicated 5 times.

Following exposure, plants showed limited visual injury symptoms with the exception of the higher treatment rates following a 24-hour exposure. Field sampling suggests the ability to maintain diquat residues over a 24-hour period is highly unlikely. Visual observations during the study did suggest that several diquat treatments were growth regulating, but the ability of the plants to recover from many of the treatments was obvious. Based on this trial, diquat would be described as a much weaker contact herbicide than carfentrazone on VM. Further work is suggested to determine conditions (light intensity, temperature, presence of epiphytes) that may impact diquat efficacy.

Endothall:

There is limited data regarding endothall (applied as Aquathol) efficacy on VM. Prior work has shown that endothall can be quite effective on Eurasian milfoil and we therefore chose rates and exposures that provided control of this plant against VM. Endothall was evaluated at 0, 1.5, and 2.5 ppm for exposure periods of 6, 18, 30, and 48 hours. Plants were evaluated over a 21-day period and then harvested. Each treatment was replicated 5 times.

Results suggest that endothall was not effective at either treatment rate through the 30-hour exposure period. These treatments were often not distinguishable from untreated controls. Plants exposed for 48 hours did show stronger injury symptoms; however,

when compared to carfentrazone, diquat, or the auxin herbicides, endothall would be considered a very weak herbicide on VM. Pending confirmation of these results, and given the numerous other effective alternatives, we would suggest no further evaluations of endothall are necessary.

Triclopyr, 2,4-D amine, and 2,4-D BEE –

Due to a similar mode of action triclopyr and 2,4-D would be expected to have similar activity on VM. To date, 2,4-D BEE has been the standard for VM control, and based on the similar characteristics between these products, we have decided to compare these products on a rate basis. VM was exposed to liquid formulations of 2,4-D amine and triclopyr and the granular formulation of 2,4-D BEE at concentrations of 0.5, 1.0, 1.5 and 2.0 ppm for 1, 2, 3, 6, 12, and 24 hours. Plants were evaluated over a 21-day period and then harvested. Each treatment was replicated 5 times.

All three products resulted in strong initial epinasty of the VM meristems. At the higher rates (1.5 and 2.0 ppm) and longer exposure periods (24 hours), all of the compounds provided nearly 90 to 100% control in these trials. While good efficacy under these use scenarios is not surprising, the objective in conducting direct comparative work is to determine if one compound is much more or less effective than the others when either marginal use rates or exposures are applied.

During this study, 2,4-D BEE consistently showed increased efficacy compared to triclopyr and 2,4-D amine. For some treatments (e.g. 1.0 ppm at 2, 3, and 6 hours), while 2,4-D amine and triclopyr provided marginal control (< 30% control), the ester formulation was highly effective at reducing biomass by greater than 80%. This result is particularly interesting given the fact that we chose to conduct these initial exposure studies knowing that not all of the 2,4-D in the BEE formulation had released from the granule. In essence, while we were comparing equivalent rates and exposures of triclopyr and 2,4-D amine, the amount of 2,4-D dissolved in the water through the first 6 to 12 hours was considerably reduced. Residues for this study have not been analyzed to date, but earlier pilot trials suggest that residues could be as much as 50% less through the first 6 hours.

Preliminary results suggest that the ester formulation of 2,4-D may have properties that increase efficacy when directly compared to either the amine formulation or to triclopyr. Additional studies are planned to confirm this observation.

Ongoing and Future Work.

Numerous confirmation studies are either ongoing or are planned. Study protocols for compounds such as fluridone, penoxsulam, and imazamox will require significant changes compared to evaluations of the faster acting compounds. We look to initiate trials of these enzyme-specific and slow acting compounds within the next couple of months.

